Detection of *Brucella melitensis* by the BacT/Alert automated system and *Brucella* broth culture

Z. Özkurt[1*], *S. Erol*[1], *M. A. Tasyaran*[1] *and A. Kaya*[2]

[1]Department of Clinic Bacteriology and Infectious Diseases, Atatürk University Medical Faculty and [2]Department of Clinical Bacteriology and Infectious Diseases, Mersin University Medical Faculty, Mersin, Turkey

[*]E-mail: zulalozkurt12@hotmail.com

This study was conducted to evaluate the ability of the BacT/Alert automated blood culture system to detect *Brucella* spp. in comparison with traditional *Brucella* broth culture. Overall, 100 (50 bone marrow and 50 blood samples) paired cultures were obtained, and 59 were positive by at least one method. The *Brucella* broth culture method detected all 59 positive cultures (100%), and the BacT/Alert system detected 30 (50.8%) ($P < 0.05$). The mean detection times for *B. melitensis* were 4.5 days in the BacT/Alert system and 5 days in *Brucella* broth culture ($P > 0.05$). There is no significant difference between the two methods with respect to growth time of the microorganism, but *Brucella* broth culture is more sensitive than the BacT/Alert system.

Keywords *Brucella* spp., blood culture, bone marrow culture, BacT/ Alert, *Brucella*

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INTRODUCTION

Brucellosis is an infectious disease endemic in Mediterranean countries. The disease has various clinical manifestations. When the clinical presentation is typical, the diagnosis of brucellosis is easy, but when non-specific manifestations are present, diagnosis becomes problematic [1,2].

The diagnosis of brucellosis is based on the isolation of bacteria from blood, bone marrow and other tissues or serology [3]. Some factors, such as the nature of its fastidious growth, its intermittent and low concentration in blood, and previous use of antibiotics, reduce the recovery of *Brucella* spp. from blood cultures [4].

In recent years, the use of automated blood culture systems has become widespread. These systems have some advantages, such as early detection of microorganisms, a decrease in contamination risk, and reduced labor [5,6].

There are few studies on the detection of *Brucella* spp. with automated blood culture systems, and experience with the use of the BacT/Alert blood culture system for the recovery of *Brucella* spp. is limited. In this study, we aimed to evaluate the ability of the BacT/Alert automated blood culture system to detect *Brucella* spp. in comparison with the traditional method using *Brucella* broth medium.

MATERIALS AND METHODS

The present study was prospectively carried out in the Ataturk University Medical Faculty Hospital, Department of Clinical Bacteriology and Infectious Diseases, between January 1996 and December 1998. Our hospital serves a population of 2.5 million inhabitants, and is the largest (1200-bed) hospital in the city of Erzurum in the Eastern Anatolian region of Turkey.

Fifty patients with suspected brucellosis, on the basis of clinical and epidemiologic features or serology, were included in this study.

The patients were divided into three groups according to the evolution of disease: acute, with symptoms for less than 2 months; subacute, with symptoms for 2 months to 1 year; and chronic, with symptoms for more than 1 year.

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Blood and bone marrow cultures and the standard tube agglutination (STA) test were performed in all patients. Ten milliliters of venous blood was drawn from all patients; 5 mL was placed in *Brucella* broth medium, and 5 mL in the BacT/Alert bottles (Organon Teknika Corp., Durham, NC, USA). Also, 6 mL of bone marrow was drawn from the sternum of all patients: 2 mL was inoculated into *Brucella* broth, and 4 mL into the BacT/Alert bottles. Subsequent blood cultures were inoculated only into the BacT/Alert system, used routinely in our hospital; these were not included in the study.

BacT/Alert cultures were incubated for 7 days; those negative at 7 days were incubated for 2 additional weeks and subcultures were made on chocolate or blood agar every 48 h.

Non-commercial *Brucella* broth (tryptone 10 g, peptamine 10 g, dextrose 1 g, yeast extract 2 g, sodium chloride 5 g, sodium bisulfite 0.1 g, supplement [cycloheximide, bacitracin, circulin and polymyxin B], and 0.25 mL of 4% sodium citrate as anticoagulant) cultures were incubated at 37 °C for 4 weeks, and subcultures were performed on chocolate or blood agar every 48 h.

Brucella isolates were identified on the basis of colony morphology, catalase and urease positivity, and agglutination with specific anti-*Brucella* sera. The *Brucella* isolates were sent to the Institute of Pendic Veterinary Control and Research for confirmation and species identification.

The STA test was used for serologic diagnosis, and a titer higher than 1/160 was considered positive. When an STA test result was negative, the test was repeated in high dilution (up to 1/2560) and with Coombs sera to detect non-agglutinating antibodies.

For statistical evaluation of the results, Student's *t*-test and Wilcoxon's Paired Signed Rank test were used.

RESULTS

Fifty patients (age range: 14–65 years) with suspected brucellosis, on the basis of clinical and epidemiologic features or serology, were included in this study. Thirty cases were considered acute, 17 subacute and three chronic. Ten patients had taken inappropriate antibiotics, 11 patients specific treatment for brucellosis in inappropriate combinations or underwent for inappropriat periods, and the remaining nine had not taken any drug before being admitted to our clinic.

The STA test was positive in 48 (96.0%) patients. In two chronic cases, the STA test was negative but bone marrow cultures were positive.

In this study, 100 pairs of samples (50 blood and 50 bone marrow) were obtained. Bone marrow and blood cultures were positive in 35 (70.0%) and 24 (48.0%) of the patients, respectively ($P < 0.005$).

Both the evolution of the disease and previous antimicrobial therapy influenced the isolation rates of *Brucella* spp. ($P < 0.05$). Culture and STA test results in relation to clinical features are summarized in Table 1.

Of 100 pairs of cultures, 59 were positive by at least one method. The *Brucella* broth method detected all of 59 (100%) positive cultures, whereas the BacT/Alert system detected only 30 (50.8%) ($P < 0.05$). Of the positive culture results, 30 were positive by both methods, and 29 positive according to *Brucella* broth culture.

Considering all bottles from each patient, positive results for 35 patients were detected by at least one method. Positive results for all 35 (100%) patients were detected by *Brucella* broth culture, and positive results for 21 (60%) of them were detected by the BacT/Alert system ($P < 0.05$).

Twenty-three of 30 positive cultures (76.7%) were detected with the BacT/Alert system within

Table 1 Culture and STA test results in relation to clinical features of the patients with brucellosis

Features of the patients	Patient *n* (%)	Positive bone marrow culture *n* (%)	Positive blood culture *n* (%)	Positive STA test *n* (%)
Acute	30 (60.0)	25 (83.3)	20 (66.6)	30 (100)
Subacute	17 (34.0)	9 (52.0)	4 (23.5)	16 (94.1)
Chronic	3 (6.0)	1 (33.0)	0 (0.0)	2 (66.6)
Previous therapy	21 (42.0)	10 (47.6)	6 (28.5)	19 (90.4)
No previous therapy	29 (58.0)	25 (86.2)	18 (62.0)	29 (100)
Total	50 (100)	35 (70.0)	24 (48.0)	48 (96.0)

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Table 2 Detection of *Brucella* spp. by *Brucella* broth and the BacT/Alert system

Method	Culture	Positive cultures *n* (%)	Patients *n* (%)	Positive marrow culture	Positive blood culture	Mean growth time (day)
Brucella broth	100[a]	59 (100)	35 (100)	35	24	5
BacT/Alert	100[a]	30 (50.8)	21 (60.0)	18	12	4.5

[a]Fifty bone marrow and 50 blood culture.

7 days, and seven of them (23.3%) were detected by subcultures after 1 or 2 weeks of incubation.

The mean times to detection of *B. melitensis* were 4.5 days (range: 32 h to 16 days) with the BacT/Alert system, and 5 days (range: 2–14 days) with the *Brucella* broth cultures ($P > 0.05$). All cultures were detected within 16 days by both methods. The results are summarized in Table 2. The mean detection times of *B. melitensis* were 4.7 days from bone marrow, and 6.8 days from blood cultures ($P < 0.05$). All isolates were identified as *B. melitensis* biotype 1.

DISCUSSION

Automated blood culture systems allow early detection of microorganisms with continuous monitoring, as well as decreases in the risk of contamination and labor [5,6]. However, the superiority of these systems over conventional culture systems is debatable in cases of slow-growing bacteria such as *Brucella* spp.

In our experience, with 59 (100%) positive *B. melitensis* blood and bone marrow cultures, 35 (100%) patients were diagnosed using the *Brucella* broth culture method, and with 30 (50.8%) positive cultures, 21 (60%) patients were also diagnosed using the BacT/Alert system.

Twenty-three of 30 (76.6%) cultures positive according to the BacT/Alert system were obtained within a 7-day incubation period, and seven (23.3%) of them were detected on subcultures after 7 days of incubation. If subcultures had not been done, 23.3% of positive cultures would not have been detected. Therefore, the standard 7-day incubation period of the BacT/Alert system is insufficient for detection of *Brucella* spp.

Casas et al. [7], using the BacT/Alert system, recovered one of five *Brucella* isolates within 3 days, and others by subsequent subculture. They suggest that the BacT/Alert system did not solve the problem of diagnosis of brucellosis, and subcultures were required. Melo-Cristino and Salgado [8] compared the VITAL system with the tryptose broth method, and found that the latter was more sensitive. Therefore, they too recommended prolongation of incubation times and subculture.

In the present study, although subcultures were done after the standard incubation period, the abilities of the BacT/Alert system and *Brucella* broth culture method to detect *B. melitensis* were still statistically different ($P < 0.05$). This finding indicates that the difficulty in detection of *B. melitensis* by the BacT/Alert system may not only be due to the short incubation time but also to the content of the medium. Gamazo et al. [9] investigated factors affecting the detection of *B. melitensis* by BACTEC NR 730. They have suggested that the bacterial concentration is low in brucella bacteremia, and sodium polyanethol sulfonate (SPS), used in automated systems, inhibits the growth of bacteria via its harmful effect on the bacterial membrane. They also suggest that the pH of the medium is unsuitable for growth of *B. melitensis* and, most important, the carbon dioxide release could be undetectable because of the peculiarities of *Brucella* metabolism. Therefore, they claimed that the BACTEC NR automated system is unsuitable for *Brucella* spp. There is no study on factors affecting the growth of *Brucella* in the BacT/Alert system, but it seems that the factors mentioned above could also be applicable.

Automated blood culture systems provide advantages in the early detection of *Brucella* spp. [10–18]. In our study, the mean detection time for *B. melitensis* was 4.5 days using the BacT/Alert system, and 5 days using the *Brucella* broth method ($P > 0.05$). Solomon and Jackson [10] detected *B. melitensis* in one case after an incubation period of only 2.8 days with the same system, and reported that the growth time of *Brucella* was 48 ± 1 h with a 10 CFU/mL concentration of bacteria. Casas et al. [7] also detected the organism in 2–3 days in five cases. Roiz et al. [11], using the BacT/Alert system, reported that, in their experience, all nine cultures obtained from five patients yielded the organism

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within 88.4 h, and in one culture of a sample from a pancreatic abscess, the growth time was 13.3 h. Sümerkan et al. [12], using the same system, reported that the mean growth time of *B. melitensis* in standard aerobic bottles was 56.2 h at 101 CFU/bottle.

Gedikoglu et al. [13] recovered 30 *B. melitensis* isolates with the BACTEC 9120 system in 4 days of incubation. Yagupsky et al. [14], using BACTEC 9240, recovered 15 of 22 *Brucella* isolates in 3 days, while Bannatyne et al. [15], also using BACTEC 9240, recovered 93% of 97 isolates in 5 days. Zimmerman et al. [16], using BACTEC NR 730, reported that the growth time of *Brucella* spp. was inversely related to the concentration of bacteria; and they found that if the concentration was 5–500 CFU/mL, *B. melitensis* growth in BACTEC NR 730 would be within 2 days, whereas 5–7 days would be required for detection if the concentration was lower than 5 CFU/mL. Ruiz et al. [17] recovered 17 *Brucella* isolates in a mean time of 3.8 days using BACTEC.

In conclusion, we observed no significant differences between the BacT/Alert and the *Brucella* broth culture method with respect to growth time of *B. melitensis*; however, a 7-day incubation period in the BacT/Alert system is insufficient for the detection of this slow-growing bacterium. To maximize detection of the organism by the BacT/Alert system, prolonged incubation time and periodic performance of subcultures are required.

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